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EFFECT OF DELTA-SLEEP PEPTIDE ON EPILEPTIC ACTIVITY DURING METRAZOL KINDLING

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Delta-sleep inducing peptide (DSIP) has an antiepileptic action when single foci of of epileptic activity and multifocal epileptic complexes are formed in the cerebral cortex of rats and cats [2]. It was considered interesting to study the antiepileptic efficacy of DSIP on other models of epileptic activity. One model of progressively increasing predisposition to epileptic activity is metrazol kindling, which arises as a result of repeated injections of subconvulsive doses of metrazol [5].

The effect of DSIP on epileptic activity in rats and mice was investigated in the study described below.

EXPERIMENTAL METHOD

Experiments were carried out on $(CBA \times C57Bl/6)F_1$ mice weighing 18-24 g and on Wistar rats weighing 180-250 g. Kindling was induced by daily (for 3 weeks) intraperitoneal injection of metrazol in a dose of 30 mg/kg. The intensity of the convulsions was estimated in points on the following scale: 0) absence of epileptic response; 1) paroxysmal twitchings; 2) clonic convulsions of the whole trunk; 3) clonic convulsions of the forelimbs, the animal raising itself on its bind limbs (kangaroo posture); 4) marked clonico-tonic convulsions with the animal falling on its side, and a phase of tonic extension; 5) repeated clonico-tonic convulsions with the animal falling on its side, terminating in death of some of the animals. The latent period of development of the first epileptic manifestations, and the mortality also were determined. DSIP in a dose of 100 μg/kg was injected intraperitoneally in physiological saline. The epileptic response of the animals was tested after 15-17 h. The action

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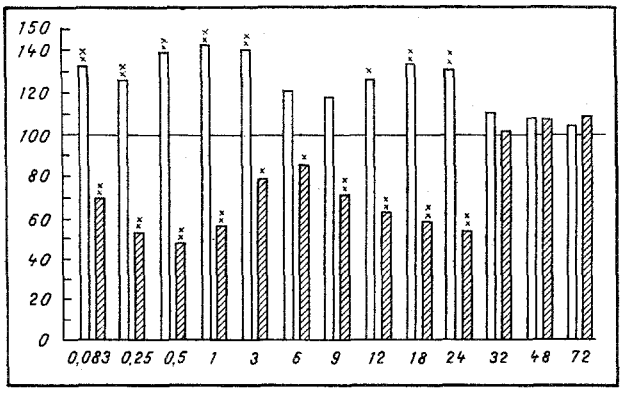

Fig. 1. Effect of time of injection of DSIP on epileptic responses in mice with metrazol kindling. Abscissa, time after injection of DSIP (in h); ordinate: unshaded columns — latent period, shaded columns — severity of convulsions (in % relative to control — 100%). Horizontal line — control, taken as 100%. Here and in Fig. 2: *p < 0.05, **p < 0.01 compared with the control.

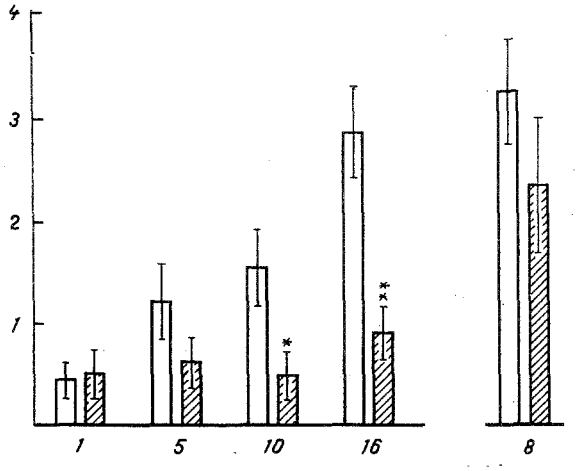

Fig. 2. Effect of DSIP on formation of increased predisposition to convulsions during kindling. Abscissa, number of metrazol injections (metrazol was injected into animals of the experimental group 30 min after DSIP and into animals of the control group 30 min after injection of physiological saline); ordinate, severity of convulsions (in points). Last two columns represent injection of metrazol 2 months after its 16th injection. Unshaded columns — control, shaded — DSIP.

of DSIP on the intensity of the convulsions was studied at different times (from 5 min to 72 h) after its injection. Naloxone (2.5 mg/kg in a volume of 0.5 ml) was injected intraperitoneally in physiological saline, 10 min before DSIP. Animals of the control groups received an injection of the corresponding volume of physiological saline. To study changes in brain electrical activity under the influence of DSIP, the rats were anesthetized with hexobarbital (100 mg/kg) and recording electrodes were implanted into the sensomotor cortex and hippocampus at coordinates (AP = +2; L = 2.5; H = 2.5) taken from the atlas [3]. Potentials were recorded by mono- and bipolar techniques on the 4-ÉÉG-3 electroencephalograph. The reference electrode was fixed in the nasal bones. The experimental results were subjected to statistical analysis by variance and nonparametric methods [4].

EXPERIMENTAL RESULTS

The action of DSIP on the latent period of the epileptic manifestations and severity of the convulsions in mice at different times after injection of DSIP was studied in the experiments of series I (Fig. 1).

TABLE 1. Effect of DSIP on Convulsions in Rats with Metrazol Kindling on 22nd Day of Injection of Metrazol (M ± m)

Experimental conditions	Severity of epileptic response, points (number of animals)					Average severity of convulsions, points
	1	2	3	4	5	
Control (metrazol, n=18)	—	5	7	5	1	3,11±0,22
DSIP + metrazol (n=12)	3	8	0*	1	0	1,92±0,23**

Legend. *$p < 0.025$, **$p < 0.01$ compared with control.

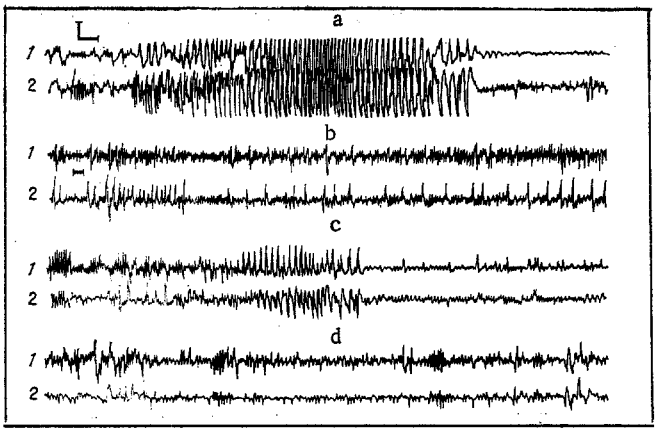

Fig. 3. Effect of DSIP on brain electrical activity of rats with induced kindling. a) 3.5 min after 21st injection of metrazol (30 mg/kg); b) 4.5 min after a; c) 4.5 min after 22nd injection of metrazol into the same rat, 30 min after intraperitoneal injection of DSIP in a dose of 100 μg/kg; d) 3 min after a. 1) Sensomotor cortex; 2) hippocampus. Calibration: 200 μV, 2 sec.

A testing injection of metrazol 5 min after injection of DSIP caused the appearance of epileptic manifestations with a latent period that was longer than in animals of the control group. The severity of the epileptic response after injection of DSIP was less than in the control. Lengthening of the latent period of convulsions and reduction of the severity of the epileptic response compared with the control were observed for 24 h after injection of DSIP. The severity of the epileptic manifestations was reduced most of all (by 50% compared with the control) 0.5-1 h, and also 24 h, after injection of DSIP. At these time intervals the latent period of the epileptic response was increased by 30-40% compared with the control. The severity of the convulsions was reduced by the greatest degree (by 15% compared with the control) 6 h after injection of DSIP; the latent period of the convulsions was increased by 18% compared with the control. Testing the epileptic manifestations in the animals 32, 48, and 72 h after injection of DSIP revealed no significant differences in the latent period and severity of the convulsions in the experimental and control groups.

In the experiments of series II, when the animals developed convulsions with an intensity of 5 points in the late stages of kindling formation, the effect of DSIP on mortality was studied. Under the influence of DSIP, death of the animals was found to be prevented: in the experimental group (eight mice) not a single animal died, whereas in the control group six of the nine mice died ($p < 0.025$).

In the experiments of series III (on rats) the effect of DSIP was studied on the development of epileptic responses at different stages of metrazol-induced kindling (Fig. 2). Metrazol was injected into animals of the experimental group 30 min after each injection of DSIP (16 injections altogether), whereas it was injected into animals of the control group 30 min after injection of physiological saline.

After the first five injections of metrazol the severity of the convulsions in animals of the experimental group was the same as in the control. The intensity of the epileptic manifestations after the 10th-16th injection of metrazol in animals of the experimental group was less than in rats of the control group. The severity of the convulsions in animals of the experimental group, induced by a testing injection of metrazol 2 months after the last injection of metrazol and DSIP, increased and was the same as in animals of the control group.

In the experiments of series IV the effect of DSIP on the intensity of epileptic responses was studied in animals subjected to 3 weeks of kindling. An additional aim was to investigate the effects of DSIP with simultaneous administration of naloxone. Under the influence of DSIP (100 μg/kg), injected 30 min before metrazol, a decrease in the severity of the epileptic manifestations and in the number of animals with generalized convulsions was observed (Table 1). The intensity of the epileptic responses after injection of naloxone was 3.25 ± 0.26 points, the same as in animals of the control group receiving metrazol alone (3.11 ± 0.22 points). EEG studies in rats of this group showed that 3-3.5 min after the testing injection of metrazol, frequent (2 Hz), high-amplitude (up to 1 mV), synchronized discharge developed in the cerebral cortex and hippocampus (Fig. 3a). Generalized convulsions lasted between 30 and 60 sec, after which epileptic activity disappeared. In the course of the EEG changes of the pattern described above, rhythmic clonic contractions of the forelimbs were observed in the rats, which got up on their hind limbs (severity of convulsions 3 points). Termination of the convulsions was accompanied by the development of postictal depression, which lasted 1-3 min. The motor activity of the rats was then restored, and clonic contractions of individual muscles and of the whole trunk were observed, accompanied by spike discharges of the EEG and by pointed waves from 200 to 800 μV in amplitude (Fig. 3b). The development of spike discharge with a frequency of 1 Hz and amplitude of 200 to 400 μV was observed 4-6 min after injection of metrazol at the 30th minute after injection of DSIP (Fig. 3c), and these discharges were accompanied by separate spasms (the severity of the convulsions 1 point). The duration of the convulsions was 20-40 sec. Later single spike potentials with an amplitude of up to 300 μV were recorded (Fig. 3d), accompanied by weak spasms.

The results are thus evidence of the antiepileptic action of DSIP in kindling induced by repeated injections of metrazol. The antiepileptic effect was expressed as an increase in the latent period of the convulsions and a decrease in the severity of the epileptic fits and mortality, and it was observed in experiments on mice at different times (from 5 min to 24 h) after injection of DSIP.

The intensity of the antiepileptic effect of DSIP varied in the course of this time. The effect was maximal after 0.5-1 h and also after 24 h, but was reduced 6-9 h after injection of DSIP. These changes in the effects of DSIP are not clear; they may be connected with experimental conditions that were not taken into account or with differences in the effects of DSIP.

The discovery of the prolonged antiepileptic action of DSIP and also the data on its rapid metabolism (half-decomposition period 9 min [6]) suggest thatunder the influence of DSIP prolonged activation of neuronal structures constituting the antiepileptic system takes place [1].

The antiepileptic effects were observed in experiments both on mice and on rats. The investigation showed that the development of generalized epileptic fits is delayed under the influence of DSIP. In animals with kindling DSIP led to a fall in the parameters of the EEG and behavioral manifestations of generalized seizure activity. The antiepileptic action of DSIP was unchanged by naloxone. Meanwhile some of the effects of DSIP, for example its sedative action, are blocked by injection of naloxone [7].

The results of this investigation and also those of previous studies [2] are thus evidence that the antiepileptic effects of DSIP are manifested both on a model of foci of epileptic activity and of their complexes, and also under conditions of generalized convulsions associated with metrazol kindling. It is an interesting fact that the antiepileptic effect of DSIP was observed when the peptide was given in a much smaller dose (10 nmoles/kg) than that of a synthetic anticonvulsant such as phenobarbital (about 10 mmoles/kg).

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STATE OF LIPID PEROXIDATION AND THE CALCIUM TRANSPORT ENZYME SYSTEM IN THE SARCOPLASMIC RETICULUM OF THE ISCHEMIC MYOCARDIUM

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KEY WORDS: heart, ischemia, lipid peroxidation, calcium uptake.

Ischemia or anoxia of the heart causes considerable impairment of the functional capacity of membranes of the sarcoplasmic reticulum (SR). In total ischemia, for instance, the ability of the membranes of SR to take up Ca^{++} is reduced after only 30 min [12]. This may be one cause of the development of postoperative heart failure after operations on the "dry" heart or after heart transplantation. It has also been shown by direct [3] and indirect [8] methods of investigation that lipid peroxidation (LPO) processes in the myocardium are intensified during ischemia, and that preliminary administration of an antioxidant improves the contractile function of the ischemic heart [9].

However, changes in concentrations of LPO products in membranes of SR and the connection between these changes and disturbance of the ability of SR to accumulate Ca^{++} in total ischemia have not been studied. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Altogether 30 experiments were undertaken on mongrel dogs. The heparinized (3 mg/kg) dogs were anesthetized with thiopental (10 mg/kg), artificial respiration was applied, and the heart was removed and kept in an incubator at 37°C. An area of myocardium of the left ventricle was excised after 15, 30, 60, and 120 min. Fragments of SR membranes were isolated, the state of their Ca^{++} transport enzyme systems was determined, lipids were extracted from the SR membranes and their concentrations of primary LPO products (diene conjugates — DC) were estimated as described previously [4]. Concentrations of total phospholipids (PL) in lipid extract from SR membranes were determined by the method in [14]. Concentrations of secondary LPO products (triene conjugates — TC) were determined spectrophotometrically by measuring the increase in optical density of the solution of lipids at 275 nm. The level of

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